

Review

How to Recruit the Correct RNA Polymerase?
Lessons from snRNA GenesOleksandr Dergai¹ and Nouria Hernandez ^{1,*}

Nuclear eukaryotic genomes are transcribed by three related RNA polymerases (Pol), which transcribe distinct gene sets. Specific Pol recruitment is achieved through selective core promoter recognition by basal transcription factors (TFs). Transcription by an inappropriate Pol appears to be rare and to generate mostly unstable products. A collection of short noncoding RNA genes [for example, small nuclear RNA (snRNA) or 7SK RNA genes], which play essential roles in processes such as maturation of RNA molecules or control of Pol II transcription elongation, possess highly similar core promoters, and yet are transcribed for some by Pol II and for others by Pol III as a result of small promoter differences. Here we discuss the mechanisms of selective Pol recruitment to such promoters.

Pol II and Pol III Transcribe Different Sets of Genes

Pol II and Pol III transcribe strikingly different shares of the genome: about 20 000 genes for Pol II versus about 700 genes for Pol III in the human genome (see GENCODE database). Pol II transcribes mRNA-encoding genes, long noncoding RNA genes, and some of the small nuclear RNA (snRNA) genes. Whereas Pol II transcription of long noncoding RNAs, which has come under renewed scrutiny in recent years, remains relatively unexplored, transcription of mRNA and snRNA genes has been studied for several decades. Genes from these last two classes differ in promoter and gene body structure (snRNA genes do not contain introns), and by the signals that direct 3' end formation of the RNA: a polyadenylation signal (most mRNA-encoding genes) or an RNA stem-loop (SL) encoding structure (most histone genes) in mRNA-encoding genes versus a 3' box in snRNA genes [1–8]. Pol III transcribes short, noncoding RNA genes encoding 5S rRNA, tRNAs, RNAs involved in processes as varied as the control of Pol II transcription elongation or the processing of other RNA molecules, and some short interspersed elements (SINES) of unknown function [9].

Pol II and III genes differ in promoter structure and termination signals. For example, Pol III terminates transcription at simple signals consisting mainly of a run of T residues [9,10], a feature that does not stop Pol II and is, indeed, often present within Pol II genes. Moreover, maturation of Pol II and Pol III products involves different processing steps and machineries [11,12]. Transcription of a Pol II gene by Pol III or vice versa is therefore expected to produce mostly nonfunctional and unstable RNAs due to improper termination and processing, although some of these molecules may be processed into short regulatory RNAs, such as microRNAs. Selective Pol recruitment is thus likely to contribute to cell energetic economy.

Pol II and Pol III Promoters

The motifs found in Pol II **core promoters** (see [Glossary](#)) of metazoan mRNA transcription units are depicted in ([Figure 1A](#), Key Figure). The most widespread are the initiator (Inr) (about 45% of promoters) [13,14] and the TATA box (less than 25% of promoters) [15]. [Figure 1B](#) shows the Pol II core promoters of mammalian snRNA genes, which are more homogenous and contain a single main core element, the proximal sequence element (PSE). The PSE is coupled to a

Highlights

With a few exceptions, Pol II and Pol III transcribe distinct sets of genes.

Pol II and Pol III promoters are generally very different in structure. The Pol II and III snRNA promoters are the exception; they are very similar to each other, a feature conserved in several species. As a result, some of these genes, notably the RPPH1 promoter, can recruit Pol II or Pol III *in vivo*.

The TATA box and PSE-type sequences (PSE in mammals and sea urchin, PSEA in *Drosophila melanogaster*, and USE in *Arabidopsis thaliana*) are the only well-defined core promoter elements that are shared by some Pol II and III promoters.

Selective Pol II or III recruitment by snRNA promoters is specified by promoter features that shift the equilibrium of competitive interactions towards recruitment of TFIIB or BRF2, and Pol II or III transcription machineries.

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regulatory element, the ‘distal sequence element’ (DSE), which binds factors that help stabilize the basal transcription machinery on the PSE [3,16].

Figure 1C–E shows the three types of Pol III core promoters. Type 1 promoters are present in the 5S genes, type 2 in most Pol III genes (i.e., tRNA genes and a few other genes), and type 3 in the spliceosomal U6 snRNA genes and another 15 or so genes, including those encoding RMRP and RPPH1 RNAs, required for maturation of rRNAs and tRNAs, respectively [9,17–20], 7SK RNA, involved in regulation of the Pol II transcription elongation factor PTEF-b [21,22], the vault RNA component of vault particles, implicated in intracellular transport, drug resistance, and more recently regulation of autophagy [23–25], and the Y RNAs, implicated in DNA replication [26]. Type 1 and 2 Pol III promoters are largely located within the RNA coding sequence (Figure 1E) [one exception being a TATA box present upstream of the transcription start site (TSS) in the *Schizosaccharomyces pombe* type 2 promoters [27], whereas type 3 Pol III promoters reside upstream of the TSS [28,29]. Type 3 Pol III promoters are, moreover, strikingly similar to the Pol II snRNA promoters, containing a DSE and a PSE [30] with, in addition, a TATA box located downstream of the PSE, centered about 28 bp upstream of the TSS [31,32] (Figure 1B,C).

Pol II and Pol III Preinitiation Complexes

Most Pol II and Pol III core promoter elements are distinct, with the exception of the TATA box and the PSE, which can be found in both classes of promoters (Figure 1). Correspondingly, although Pol II and Pol III preinitiation complexes (PICs) are similar in topology and architecture, only two basal TFs can be found in both, the TATA box-binding protein (TBP) and the snRNA activating protein complex {SNAPc; also known as PSE transcription factor (PTF) [33]}, which binds to the PSE (Figure 1B,C) [34–36]. These factors perform, however, fundamentally different functions, with TBP playing a general role in transcription and SNAPc serving to recruit TBP and other factors to PSE-containing promoters.

TBP is involved in transcription by Pol I, II, and III as part of, respectively, the selectivity factor 1 (SL1), TFIID, and TFIIB complexes, which contain different TBP-associated factors (TAFs) [37,38]. Thus, although TBP was discovered as a TATA box-binding factor, it is part of PICs assembled at TATA-less promoters, which in mammals include Pol I, and the large majority of Pol II and Pol III promoters. At such promoters, TBP is recruited through protein–protein and protein–DNA interactions involving the TAFs [39,40]. For example, the DNA binding function of TBP is not required for transcription from TATA-less Pol II mRNA promoters [41], where TBP is recruited through interactions of different TFIID TAFs with the Inr, the downstream promoter element (DPE), or the motif ten element (MTE; Figure 1; see [42,43] and references therein).

If most promoters are TATA-less, why is TBP so generally present in PICs? The answer may be in its ability to recruit, through protein–protein contacts, the members of the TFIIB family of TFs (Box 1), namely TAF1B, TFIIB, or for Pol III the TFIIB-related factors BRF1 or BRF2 as well as another factor, BDP1. These proteins associate directly not only with TBP but also, highly selectively, with Pol I, Pol II, or Pol III [37,44–46]. The TBP-mediated incorporation of TAF1B, TFIIB, or one of the BRFs together with BDP1 within the assembling PIC is thus a decisive step in the recruitment and selection of the correct Pol. The role of basal DNA binding factors such as TFIIA, which stabilizes TBP and TFIIB on Pol II promoters (Figure 1A,B) [40,47], or TFIIIA and/or TFIIIC, which form the platform allowing recruitment of TBP together with BRF1 and BDP1 on type 1 and 2 Pol III promoters (Figure 1D,E) [35,48], is thus largely to favor the recruitment of TBP associated with the correct TFIIB family member. Consistent with TFIIIA and TFIIIC being dispensable for Pol III transcription, a combination of a TATA box and TBP, BRF1, and BDP1 is sufficient to direct Pol III transcription *in vitro* [49,50]. Further, the human C-Myc promoter and various TATA box containing core Pol II promoters can drive both Pol II and Pol III

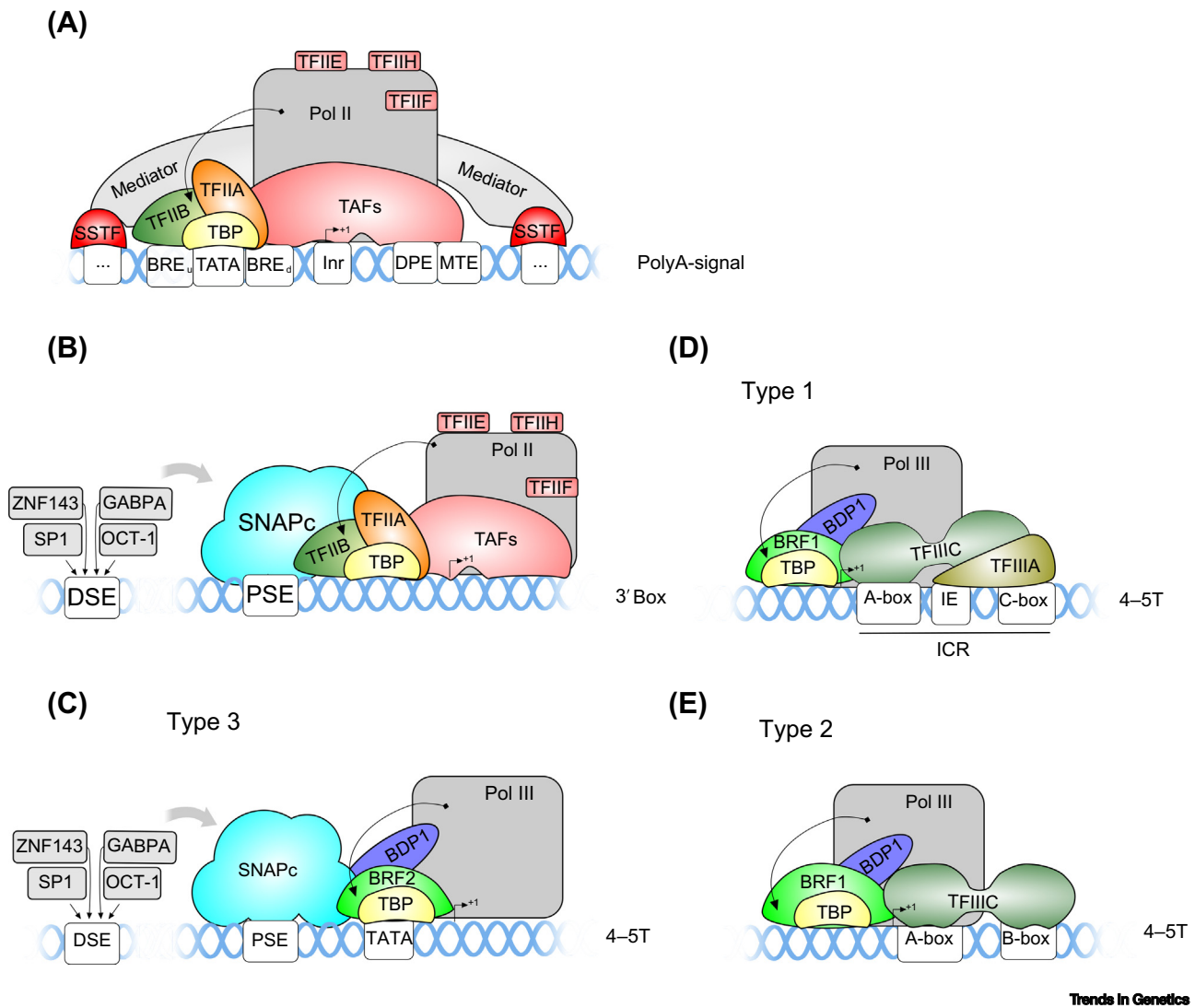
Glossary

Core promoter: the promoter region capable of recruiting, *in vitro*, the basal transcription machinery, comprising the basal TFs and the Pol. It is generally located within 100 base pairs upstream and downstream of the TSS.

Regulatory elements: DNA sequences that can be located far away from the TSS and that contain binding sites for SSTF that activate or repress transcription from the core promoter. SSTF binding can be cooperative with the binding of basal TFs.

Key Figure

Pol II and III Promoters and Components of the Corresponding PICs



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Figure 1. (A) Generic Pol II mRNA promoter. Different core promoters contain different combinations of the depicted motifs. Transcription factor IID (TFIID), composed of TATA box-binding protein (TBP) and TBP-associated factors (TAFs), is recruited through TBP–TATA box interactions and/or TAFs interactions with the initiator (Inr), the downstream promoter element (DPE), or the motif ten element (MTE). In certain promoters, TFIIB recognition elements located either upstream (BRE_u) or downstream (BRE_d) of the TATA box help recruit TFIIB. TFIIA binds to and stabilizes the TBP–DNA complex. TFIIB interacts directly with Pol II. Various **regulatory elements** recruit site-specific transcription factors (SSTF) that can act on the preinitiation complex (PIC) through contacts with the general transcription machinery or with subunits of the mediator, a large complex with both activating and repressing modules (see [97] for a review). TFIIF, TFIIE, and TFIIH are required for transcription initiation (see [43] for a review). (B) Pol II small nuclear RNA (snRNA) promoter. The core promoter consists of the proximal sequence element (PSE), which recruits the snRNA activating protein complex (SNAPc). The PIC contains, in addition, TBP together with some of the TFIID TAFs, TFIIA, and TFIIB, which recruits Pol II, TFIIF, TFIIE, and perhaps TFIIH [54,98,99]. Factors binding to the distal sequence element (DSE) activate transcription, at least in part, by stabilizing the PIC on the core promoter. (C) Type 3 Pol III promoter. The promoter contains a PSE and DSE interchangeable with the PSE and DSE of Pol II snRNA promoters and, in addition, a TATA box. The PIC contains

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Box 1. The TFIIB Family of Transcription Factors

All known multisubunit cellular RNA Pols require accessory protein factors for specific recruitment to their cognate promoters, from bacterial Pol, which uses σ factors, to archaeal and eukaryotic Pols, which require several factors, among them members of the TFIIB family of transcription factors. Remarkably, bacterial σ factors, archaeal TFB, and eukaryotic TFIIBs are in fact structural homologs and share the property of binding directly to the Pol with part of the protein very close to the catalytic core [101]. However, whereas the σ factor binds directly to promoter sequences, efficient binding of TFIIB family members to promoter core sequences requires the help of other proteins such as TBP and/or TBP-associated factors (TAFs), and TFIIB can be seen as bridging DNA-bound TBP/TAFs with Pol II. Thus, although TFIIB, for example, recognizes the BRE_u and BRE_d elements present upstream and downstream of the TATA box in some promoters, these interactions are not sufficient for recruitment in the absence of TBP. TFIIB family members retain, however, other properties of σ factor, such as stimulation of RNA chain synthesis [102].

transcription in injected *Xenopus* oocytes and HeLa cell extracts, the choice of the Pol being determined by the ratio of template to factors [51–53]. This confirms that a TATA box can assemble both Pol II and Pol III PICs and suggests that specific Pol recruitment is determined by competitive binding of basal TFs to the template. As described later, SNAPc is an interesting assembly factor that unlike TFIIA, TFIIB, or TFIIC, can promote the recruitment of TBP and either BRF2 or TFIIB, depending on the presence or absence of a TATA box [54] (see Box 2).

In addition to the basal TFs TBP and SNAPc, the Pol II and III transcription machineries can share regulatory factors such as Oct-1, Sp1, GABPA, or ZNF143 ([55] and references therein), which can bind to the DSEs of Pol II and III PSE-containing promoters as well as to mRNA Pol II promoters, or Myc [56], Rb [57,58], NF- κ B [59], and p53 [60], which are recruited to Pol II and III promoters through interactions with DNA elements or the basal transcription machinery. These factors contribute to transcription regulation, but not to the selectivity of Pol recruitment.

PSE-Containing Promoters: Structure and Features in Different Species

Curiously, although the promoter structures themselves vary in different species, the similitude of snRNA Pol II and III promoters has been conserved in several species of animals and plants. Indeed, the only known example so far of a gene transcribed by different Pol in different eukaryotes is the U3 snRNA gene. This gene is transcribed by Pol III in monocots and dicots, but by Pol II in vertebrates and lower eukaryotes including yeast [61], suggesting a change from Pol II to Pol III specificity in an angiosperm ancestor of monocots and dicots [62].

The first demonstration that the specificity of Pol II and III snRNA promoters could be easily altered was provided with manipulations of the *Xenopus tropicalis* U6 snRNA gene [28]. Precise mutagenesis in the mammalian genes then showed that a TATA box, located between the PSE and the TSS, is a dominant element triggering Pol III over Pol II specificity, whereas the PSEs contribute little to Pol specificity (Figure 2) [31]. The position of the TSS is defined largely by the PSE for Pol II, and the TATA box for Pol III genes [31].

In *Drosophila melanogaster*, the core promoter of snRNA genes consists of a proximal sequence element A (PSEA) sequence, slightly different in the Pol II and Pol III promoters, followed by a proximal sequence element B (PSEB) sequence in the Pol II, and by a TATA box in the Pol III promoters (Figure 2) [63,64]. The key determinant of Pol specificity is not presence or absence of the TATA box, but rather the precise sequence of the PSEA element. The spacing between the PSEA and PSEB, or the PSEA and the TATA box, is crucial for efficient transcription but does not contribute

SNAPc bound to the PSE, TBP bound to the TATA box, as well as the TFIIB-related factor 2 (BRF2) and another factor BDP1, which recruit Pol III. TBP, BRF2, and BDP1 form the BRF2–TFIIB complex. (D) Type 1 Pol III promoter. The promoter is gene-internal and consists of an internal control region (ICR) composed of A and C boxes separated by an intermediate element (IE). The ICR recruits the zinc finger protein TFIIB, followed by the TFIIC complex. The BRF1–TFIIB complex, composed of TBP, the TFIIB-related factor 1 (BRF1), and BDP1, can then join the PIC and recruit Pol III. (E) Type 2 Pol III promoter. The promoter is gene-internal and consists of A and B boxes, which directly recruit TFIIC. The PIC then assembles like on type 1 Pol III promoters.

Box 2. SNAPc in Different Species

The specific features of snRNA promoters that dictate which of either Pol II or Pol III is recruited are different in different species. Yet, at least in the species where it has been characterized, namely human and mouse, *Drosophila melanogaster*, and trypanosomes, the PSE-binding factor turns out to be conserved (see [103] for a review). Mammalian SNAPc is composed of five subunits: SNAP190, SNAP50, SNAP45, SNAP43, and SNAP19; SNAP190 constitutes the backbone of the complex and contains a four-and-a-half Myb repeat DNA-binding module [36]. A subcomplex consisting of a truncated SNAP190, SNAP19, SNAP43, and SNAP50, referred to as mini-SNAPc, is competent for PSE binding and *in vitro* transcription [16,104]. Interestingly, *Drosophila* SNAPc is very similar to mammalian mini-SNAPc, with a large subunit corresponding to the truncated SNAP190 of mini-SNAPc and smaller subunits resembling SNAP43 (DmSNAP43) and SNAP50 (DmSNAP50) [91].

In trypanosomes, there is only one snRNA synthesized by Pol II, the so-called SL RNA, a short RNA involved in the processing of polycistronic RNAs into individual mRNAs by trans-splicing [105,106]. SL RNA transcription requires trypanosome SNAPc (tSNAPc), whose three subunits clearly resemble the core SNAP190, SNAP50, and SNAP43 human SNAPc subunits [103,107]. tSNAPc does not appear, however, to be required for transcription of any trypanosome Pol III genes [108].

to the selectivity of Pol recruitment. In this case the position of the TSS is defined by the PSEA for both Pol II and Pol III genes [63,65].

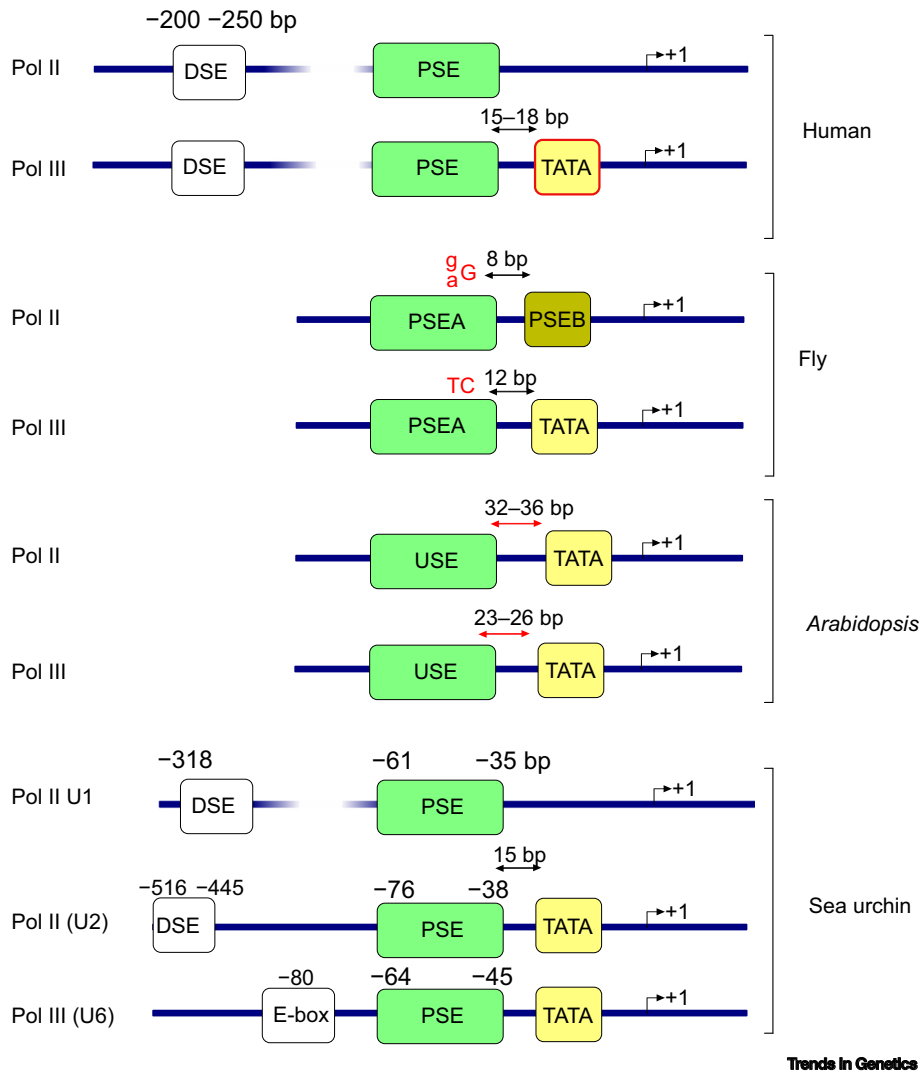
In *Arabidopsis thaliana*, Pol II and Pol III transcription of snRNA genes requires the same two basal promoter elements: an 'upstream sequence element' (USE) and a TATA box [66]. In maize, an additional element, monocot-specific promoter element (MSP), located upstream of the USE, is required for efficient transcription [67]. Pol specificity is determined by the spacing between the USE and TATA box: 32–36 bp spacing specifies Pol II transcription whereas 23–26 bp spacing specifies Pol III transcription (Figure 2) [61,68]. A small deletion between these two elements may thus be at the origin of the change in Pol specificity in plant U3 snRNA genes.

snRNA promoters have been examined in other species, but in these cases the elements determining specific Pol II recruitment have not been identified. Thus, in zebrafish, some of the U6 snRNA promoters contain a putative DSE with a CCAAT sequence as well as clearly recognizable PSE and TATA box [69]. In chicken, the Pol II U1, U2, U4, and Pol III U6 snRNA genes contain virtually identical PSEs and DSEs [70–72]. In sea urchin, snRNA genes have functional DSE and PSE equivalents that differ in different snRNA genes and have no similarity to the mammalian elements [73–75]. The PSEs in the sea urchin Pol II and Pol III genes are interchangeable and form similar DNA–protein complexes as determined by electrophoretic mobility shift assays (EMSAs), suggesting that like the PSE in other species (see later), they bind the same factors [76]. Curiously, both the Pol II U2 and the Pol III U6 snRNA promoters have, in addition to the PSE, an essential TATA box (Figure 2) [73], a feature not seen in Pol II snRNA promoters of other animal species.

Molecular Mechanisms of Selective Pol Recruitment to PSE-Containing Promoters

Current knowledge of PIC assembly at snRNA promoters results mainly from *in vitro* studies of partial PIC reconstitution, *in vitro* transcription assays, and genome-wide studies of specific TFs and Pol localization. Selective Pol recruitment to PSE-dependent genes is best understood in mammals and *Drosophila*. A key step in both systems is recognition of the PSE by SNAPc Box 2. At human promoters, where the PSE is coupled to an upstream DSE, often containing an OCT-1 binding site, OCT-1 and SNAPc bind cooperatively to their respective binding sites thanks to a protein–protein contact between the two factors that relieves autoinhibition of SNAPc DNA binding [77]. The assembly of a Pol II or Pol III specific PIC is, however, determined by specific features of the core promoter.

Pol II PICs appear to assemble differently at PSE-containing (and TATA-less) promoters than at mRNA promoters. In the latter case, assembly of the PIC involves the recruitment of the TFIID

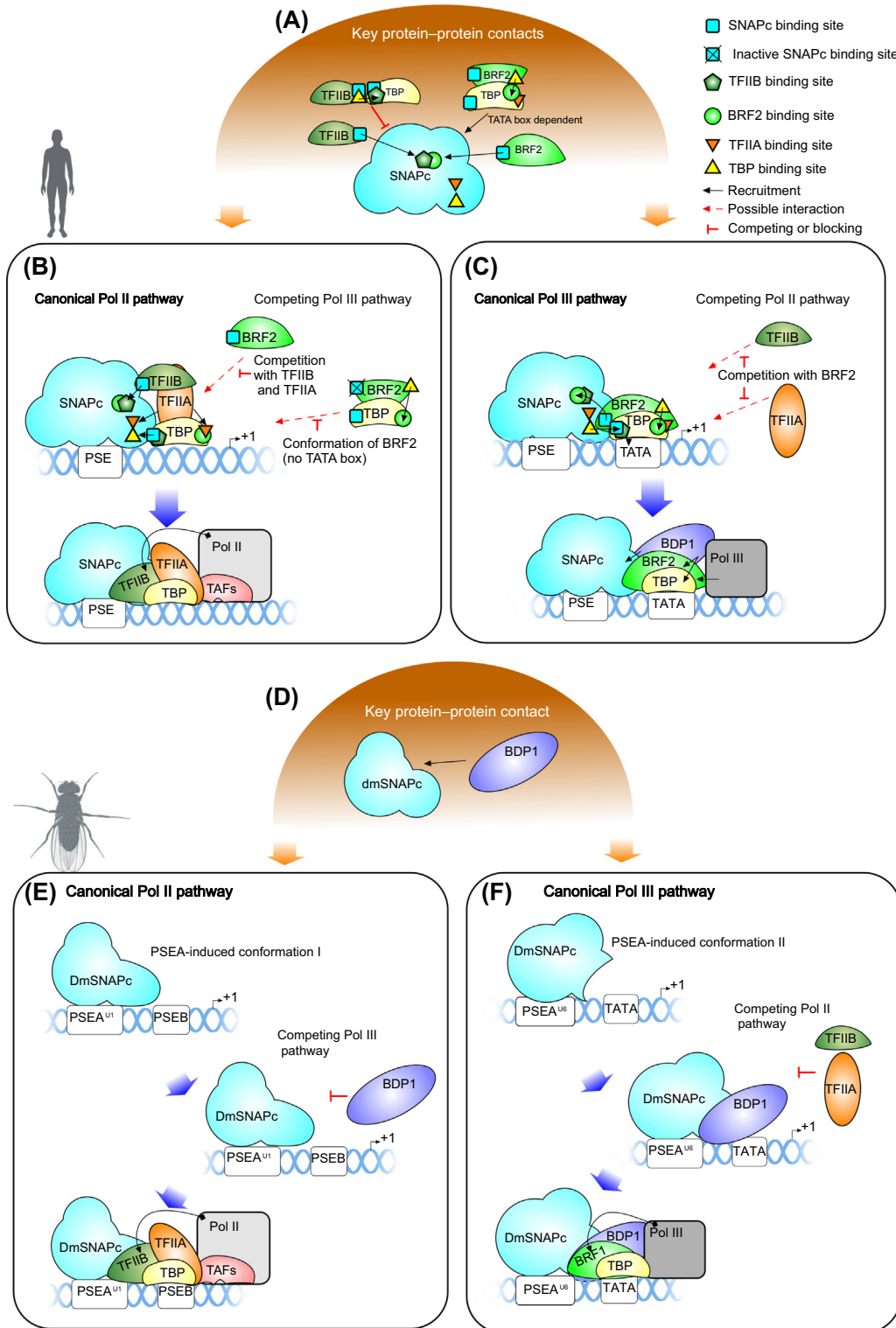


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Figure 2. Structure of Human, *Drosophila*, *Arabidopsis*, and Sea Urchin PSE-Containing Promoters. The elements that dictate Pol specificity are indicated in red. The determinants of Pol specificity in the sea urchin promoters are not known. Abbreviations: DSE, distal sequence element; E-box, enhancer box; PSE, proximal sequence element; PSEB, proximal sequence element B; USE, upstream sequence element.

complex, which binds to the TATA box and/or the Inr element through contacts with TBP and with TAFs, respectively (Figure 1A; [43] and references therein). TFIIA allows efficient TBP–DNA interaction by displacing the N-terminal portion of TAF1 from the concave DNA-binding surface of TBP, and by stabilizing the TBP–TATA box complex [40]. TFIIB is recruited via a direct interaction with TBP involving the C-terminal stirrup of TBP and the TFIIB core domain [78], an event that then allows the recruitment of Pol II [45,79]. Thus, in mRNA promoters, the TATA box cooperates with other core promoter motifs to recruit the TBP-containing complex TFIID [15,43].

At PSE-containing promoters, TFIIB is recruited by protein–protein contacts with SNAPc. BRF2 can also bind directly to SNAPc, but the binding of TFIIB and BRF2 is mutually exclusive,



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suggesting that these two proteins interact with overlapping SNAPc surfaces (Figure 3A, olive green pentagon and light green circle on SNAPc). Remarkably, however, the binding of TFIIIB to SNAPc is strongly decreased if TFIIIB is first complexed with TBP. Indeed, in this case, (i) the TFIIIB surfaces used to bind to SNAPc and to TBP (Figure 3A, turquoise square and yellow triangle on TFIIIB) overlap, and (ii) the TBP surfaces used to interact with SNAPc and with TFIIIB (Figure 3A, olive green pentagon and turquoise square on TBP) overlap. This latter observation strongly suggests that on Pol II PSE-containing promoters, TBP and TFIIIB are recruited separately from each other (Figure 3B, upper drawing), unlike at TATA box containing mRNA promoters. Nevertheless, it seems likely that after separate recruitment of TBP and TFIIIB, the proteins rearrange inside the maturing complex to reach a final conformation close to that achieved at Pol II mRNA promoters (Figure 3B, lower drawing) [54].

TFIIA can directly contact SNAPc (Figure 3A,B, upper drawing, orange triangle on SNAPc), which strengthens the SNAPc–TBP interaction [54]. Importantly, TFIIA contributes to the specific assembly of a Pol II PIC at PSE-containing TATA-less promoters by competing with BRF2, but not with TFIIIB, for binding to TBP, consistent with two overlapping surfaces of TBP binding to BRF2 and TFIIA (Figure 3A,B, upper drawing, light green circle and orange triangle on TBP); [80]. Thus at TATA-less promoters, both TFIIIB and TFIIA, the two canonical Pol II TFs, contribute to shifting the equilibrium towards Pol II PIC assembly [54].

Another effect possibly contributing to favoring Pol II recruitment at TATA-less promoters is a property of TFIIIB. TFIIIB exists in two conformations [81–83]: in its ‘closed’ conformation, a charged cluster domain (CCD) located in its N-terminal region occludes its core domain and thus prevents efficient SNAPc binding; in its ‘open’ conformation, the intramolecular binding of the CCD to the core domain is disrupted. A truncated TFIIIB lacking the CCD has much higher affinity for SNAPc than full-length TFIIIB, suggesting that the same would be true for TFIIIB in an open conformation [54]. Such a large difference in affinity is not observed in a comparison of full-length BRF2 and BRF2 core domain, and importantly, truncated TFIIIB has higher affinity for SNAPc than BRF2 [54]. Thus, in the presence of transcriptional activators bound to the DSE and perhaps causing TFIIIB to adopt an open conformation, TFIIIB would have a competitive advantage over BRF2 in binding to SNAPc when the PSE is the sole core promoter element [54].

At mammalian PSE and TATA box-containing promoters, Pol III specificity is triggered by simultaneous recognition of the PSE by SNAPc and the TATA box by TBP (Figure 3C). The distance separating the PSE and the TATA box is relatively constant in different promoters, and an increase

Figure 3. Assembly of Pol II or III PICs at PSE-Containing Promoters. (A) Key interactions (black arrows) that trigger selective Pol II or III preinitiation complex (PIC) assembly in mammals. Transcription factor IIB (TFIIIB) and TFIIIB-related factor 2 (BRF2) can both bind to small nuclear RNA activating protein complex (SNAPc), but in a mutually exclusive manner, suggesting they bind overlapping SNAPc surfaces. A TFIIIB–TBP complex cannot interact with SNAPc (red line), because of overlaps between: (i) the TATA box-binding protein (TBP) and SNAPc binding surfaces in TFIIIB, and (ii) the TFIIIB and SNAPc binding surfaces in TBP. A BRF2–TBP complex can interact with SNAPc bound to the proximal sequence element (PSE) only upon binding to a TATA box located at the correct distance downstream of the PSE. In this complex, the TFIIA binding site on TBP is occluded by BRF2. (B) Assembly of a mammalian Pol II PIC. Upper drawing: TBP and TFIIIB are recruited separately to the SNAPc–PSE complex via their SNAPc-interacting surfaces. TFIIA can bind to SNAPc and to TBP, whose TFIIA binding site is not occluded. BRF2 cannot join the complex because its binding site on SNAPc is occluded by TFIIIB and its binding site on TBP is occluded by TFIIA. As there is no TATA box, the SNAPc binding site of BRF2 in the BRF2–TBP complex is not available for binding to SNAPc, probably because of inadequate conformation. Lower drawing: the complex may rearrange to acquire a TBP–TFIIIB–TFIIA conformation close to that in mRNA promoters. (C) Assembly of a mammalian Pol III PIC. Upper drawing: in the presence of both a PSE and a TATA box, the TBP–BRF2 complex acquires a conformation such that the SNAPc binding site on BRF2 is available, and thus TBP and BRF2 both contact SNAPc. BRF2 occludes both the TFIIIB binding site on SNAPc and the TFIIA binding site on TBP, preventing these proteins from joining the complex. Lower drawing: the incorporation of BDP1 into the complex allows efficient transcription initiation by Pol III. In panels B and C, dashed red arrows indicate competing interactions and red solid lines indicate inhibitory mechanisms. (D) Key interaction that triggers selective Pol II or III PIC assembly in *Drosophila melanogaster*. BDP1 directly binds to SNAPc, and this interaction is conditioned by a SNAPc conformation dictated by the specific proximal sequence element A (PSEA) sequence. (E) Assembly of a *D. melanogaster* Pol II PIC. The PSEA sequence in the Pol II U1 snRNA promoter (PSEA^{U1}) causes DmSNAPc to adopt a conformation in which the BDP1 binding site is not available, allowing Pol II PIC assembly. (F) Assembly of a *D. melanogaster* Pol III PIC. On the PSEA sequence of the Pol III U6 snRNA promoter (PSEA^{U6}), DmSNAPc adopts a conformation which allows BDP1 binding, presumably creating a platform for BRF1 and TBP recruitment [91,92,100]. This in turn shifts the equilibrium to Pol III PIC assembly.

of 3–4 bp or more strongly diminishes SNAPc–TBP complex formation and transcription [34], consistent with the observed cooperative binding of SNAPc and TBP to the U6 promoter [84]. BRF2 recruitment is then favored by several cooperative interactions (Figure 3C, upper drawing): with SNAPc, TBP, and DNA. BDP1 further stabilizes the PIC by binding to the BRF2–TBP heterodimer, to SNAPc, and to DNA just upstream of the TATA box (Figure 3C, lower drawing) [85]. Thus, efficient BDP1 recruitment occurs after Pol specificity has been determined by recruitment of BRF2, and in this sense does not play a role in determination of Pol specificity [54].

The most important factor for favoring Pol III recruitment at TATA-containing promoters is probably TBP. Indeed, *in vitro*, in the absence of TBP, the SNAPc–BRF2 binary complex is not specific with regard to promoter sequence, it can be assembled at any PSE containing promoter, whether a TATA box is present or not (Figure 3A) [54]. Specificity arises upon addition of TBP, which enhances BRF2 association at TATA box containing promoters (Figure 3C), and most importantly, prevents BRF2–SNAPc interaction in the TATA-less context (Figure 3B, upper drawing) [54]. This is consistent with the hypothesis that, in the context of a PSE and a downstream TATA box, SNAPc, TBP, and BRF2 form a complex in which BRF2 has a different conformation than when in a heterodimer with TBP off the DNA (Figure 3A,B, upper drawing). BRF2 requires a region referred to as ‘the arch’ to assemble with SNAPc and TBP on PSE-containing and TATA-containing probes in EMSAs [80]. Perhaps the arch adopts the correct conformation only when BRF2 is in a complex with TBP bound to the TATA box downstream of the PSE (Figure 3B,C, upper drawings, turquoise square on BRF2).

Since BRF2, when not associated with TBP, can bind to DNA-bound SNAPc *in vitro* provided that SNAPc is not bound by TFIIIB, and since BRF2 specifically associates with Pol III, one could imagine that a SNAPc–BRF2 complex might assemble on TATA-less snRNA promoters and erroneously recruit Pol III. However, only traces of BRF2, and no BDP1, were detected at such promoters in cultured cells by chromatin immunoprecipitations [54]. Thus, a BRF2–SNAPc complex rarely assembles *in vivo* at TATA-less snRNA promoters and is probably transcriptionally inactive as it does not detectably recruit BDP1 [49,50,85].

In *D. melanogaster* (Dm), the PSEAs of Pol II and III promoters carry essential pol specificity information [63,86–88], and are thus not interchangeable, unlike the PSEs in the mammalian system. Careful crosslinking and EMSA studies have shown that DmSNAPc adopts alternative conformations dictated by the type of PSE it is associated with (Figure 3E,F) [89,90]. One of its subunits, DmSNAP43, contacts approximately 20 bp of DNA downstream of the Pol II U1 PSEA, but only about 5 bp downstream of the Pol III U6 PSEA [91]. The specific DmSNAPc conformation is sensed by BDP1, which associates efficiently with DmSNAPc bound to a U6 PSEA (Figure 3F) but not a U1 PSEA (Figure 3E) [92]. Conceivably, the BDP1 binding surface of DmSNAPc is occluded or inappropriately folded in the U1 PSEA-bound context. Formation of a PSEA-bound DmSNAPc–BDP1 complex presumably creates a platform for subsequent TBP, BRF1, and Pol III recruitment (Figure 3F). On Pol II promoters, the inability of BDP1 to bind DmSNAPc associated with a U1-type PSEA might leave DmSNAPc interfaces vacant for the binding of TFIIIB and other Pol II-specific factors (Figure 3E).

The mechanisms of selective Pol recruitment operating in mammals and fly appear different, with the sequence-induced different DmSNAPc conformations being critical in the fly. However, although the mammalian Pol II and Pol III PSEs are interchangeable, chromatin immunoprecipitation experiments with antibodies directed against different SNAPc subunits reveal striking shifts in tag densities, with two SNAPc subunits displaying peaks closer to the TSS in Pol III than in Pol II promoters [55]. Thus, like in flies, SNAPc adopts different conformations on Pol II and III promoters, but the different conformations most likely result from protein–protein contacts with

TATA-bound TBP and BRF2 in the Pol III promoters rather than from the PSE sequence *per se*. The same general principle may apply in plants, where the key determinant of Pol specificity is spacing between the USE and the TATA box. Here also, the SNAPc conformation may be dictated by different protein–protein contacts with TATA-bound TBP imposed by the location of the TATA box relative to the USE. The key principles leading to specific Pol II or III recruitment on mammalian PSE-containing promoters are stepwise recruitment, rather than recruitment as a complex, of TBP and TFIIB on Pol II PSE-containing promoters, and highly favored interaction of a BRF2–TBP complex over a TFIIB–TBP complex with PSE-bound SNAPc in the context of a TATA box containing promoter. Perhaps, then, regardless of promoter differences in different species, these key principles also hold for PSE-containing promoters in *Drosophila* and other species.

Dual Pol II and Pol III Transcription

The high similarity of Pol II and Pol III PSE-containing promoters raises the possibility of their widespread dual occupancy *in vivo*. Yet, although the U6, 7SK, and RPPH1 promoters can direct luciferase or CRISPR-Cas9 (clustered regularly interspaced palindromic repeats-CRISPR associated protein 9) expression by Pol II transcription from transfected plasmids [93,94], only the RPPH1 promoter is convincingly occupied by Pol II and Pol III within the genomic context [55]. This promoter displays the longest distance separating the PSE and the TATA box. Decreased Pol recruitment selectivity could thus result from weaker cooperative binding of SNAPc and TBP to their respective targets, which in turn would allow occupancy by either SNAPc alone bound to the PSE, leading to recruitment of TFIIB as described earlier (Figures 1B and 3B), or to a TBP–TFIIB complex bound to the TATA box leading to a mRNA type Pol II transcription initiation complex (Figure 1A). The first possibility seems more likely, as transfected RPPH1 promoter–luciferase constructs direct a Pol II TSS 8 bp upstream of the Pol III TSS [93], that is, at a distance downstream of the PSE typically observed at Pol II snRNA promoters and at a U6 promoter with a mutated TATA box [31]. Indeed, efficient expression of protein coding genes transcribed from the mouse Pol II U1 snRNA promoter has been observed in transfected cells, an observation consistent with the known capping mechanism of Pol II snRNAs [95], which first acquire the conventional 7-monomethylguanosine cap recognized by the translational machinery. The further methylation leading to a trimethyl G cap occurs after association with small nuclear ribonucleoproteins (snRNPs), a step that will not occur for an RNA lacking a snRNA sequence [96].

Concluding Remarks

Pol II and III promoters are widely different in structure except for the Pol II snRNA promoters and the Pol III type 3 promoters, which share common promoter elements and whose Pol specificity can be easily altered with small promoter sequence changes. The resemblance of these Pol II and III promoters is conserved in different species, even when the promoter elements themselves are not, and so too is the use of a common and specialized SNAPc-type TF. Understanding whether this conserved resemblance of Pol II and Pol III snRNA promoters affords a biological advantage will be a challenge. A possibility is a need for coregulation, which seems natural in the case of certain snRNA genes whose products function together, for example, in pre-mRNA splicing, but whose role in other cases will be difficult to establish. Indeed, RNAs transcribed from PSE-containing promoters function at numerous steps of gene expression, whose links are not fully understood.

PSE-containing Pol III promoters have several properties that make them highly suitable for *in vivo* expression of short RNAs, and indeed they are commonly used for such purpose. They are strong promoters, they are located upstream of the transcribed region and thus do not constrain the sequence of the RNA, and they fire a polymerase that terminates transcription at simple runs

Outstanding Questions

What are the 3D structures of SNAPc in Pol II and Pol III PICs?

What are the 3D structures of DmSNAPc bound to a PSEA^{U1} and DmSNAPc bound PSEA^{U6}? Are the structural changes similar to those observed with mammalian SNAPc in Pol II and Pol III PICs?

What are the molecular mechanisms leading to specific Pol II or III recruitment in different species such as sea urchin or *Arabidopsis thaliana*? Do they suggest some general principles applicable to all snRNA-type genes?

Are the positions of TBP and TFIIB in the final Pol II PIC assembled on a PSE different from their positions in a Pol II PIC assembled on a TATA box?

Are the 'open' and 'closed' conformations of TFIIB playing a role in Pol II snRNA gene transcription?

Are the Pol II and Pol III PSE-containing promoters coregulated? Under which circumstances?

Is transcription of the RPPH1 gene, and perhaps other genes with PSE-containing promoters, by both Pol II and Pol III of any functional significance?

Why are some snRNAs transcribed by Pol II rather than Pol III, like most short RNAs? Is it because Pol III would often recognize the internal Sm binding site, required for assembly of the RNA with Sm proteins to form an snRNP, as a termination signal, due to it containing a short run of T residues?

of T residues. Even if dual transcription of PSE-containing promoters by Pol II and Pol III appears to be the exception rather than the rule, it seems important when such promoters are used for therapeutic purposes, to optimize not only transcription levels by Pol III, but also to ensure absence of transcription by Pol II, as such transcription might lead to longer RNAs containing potentially harmful sequences (see Outstanding Questions).

Acknowledgments

We thank Dr M.C. Gambetta and P. Cousin for critical comments on this review. We also thank the reviewers for their comments, which improved the manuscript. This work was funded by the University of Lausanne and grant 31003A_169233 from the Swiss National Science Foundation.

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